## SECUENCE LISTING

<110> Moyle, William R. Xing, Yongna 5 <120> Protein Knobs <130> 268/279-RWJ-01-40 10 <140> 60/345,283 <141> 2001-11-08 <160> 56 15 <170> PatentIn version 3.1 <210> 1 <211> 92 <212> PRT 20 <213> Homo sapiens <400> 1 Ala Pro Asp Val Gln Asp Cys Pro Glu Cys Thr Leu Gln Glu Asn Pro 25 Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys 30 Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Lys Lys Thr Met Leu Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser 35 Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser 40 <210> 2 <211> 92 45 <212> PRT <213> Artificial Sequence <223> hCG alpha-subunit with Cys substituted for Gln5 50 <400> 2 Ala Pro Asp Val Cys Asp Cys Pro Glu Cys Thr Leu Gln Glu Asn Pro 55 Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Lys Lys Thr Met Leu 60 Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser 55

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2/41

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    Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser
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    Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser
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    Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser
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     Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Lys Lys Thr Met Leu
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     Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser
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     Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser
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Phe Ser Cys Ala Tyr Pro Thr Pro Leu Arg Ser Lys Lys Thr Met Leu 35 40 45

15

Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser 50 55 60

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Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr 65 70 75 80

Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser 85 90

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**422** 

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45 Phe Ser Arg Ala Cys Pro Thr Pro Leu Arg Ser Lys Lys Thr Met Leu 35 40 45

50

Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser 50 55 60

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Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr 65 70 75 80

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Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser

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Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser

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•					•											
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60	Val	Gln 50	Lys	Asn	Val	Thr	Ser 55	Glu	Ser	Thr	Cys	Суз 60	Val	Ala	Lys	Ser

Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr

65 70 75 80 Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser 5 85 <210> 15 <211> 92 10 <212> PRT <213> Artificial Sequence <220> <223> hCG alpha-subunit with Cys substituted for Arg42 15 <400> 15 Ala Pro Asp Val Gln Asp Cys Pro Glu Cys Thr Leu Gln Glu Asn Pro 20 Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys 25 Phe Ser Arg Ala Tyr Pro Thr Pro Leu Cys Ser Lys Lys Thr Met Leu 40 30 Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser 50 55 Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr 35 70 Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser 85 40 <210> 16 <211> 92 <212> PRT 45 <213> Artificial Sequence <220> <223> hCG alpha-subunit with Cys substituted for Ser43 50 <400> 16 Ala Pro Asp Val Gln Asp Cys Pro Glu Cys Thr Leu Gln Glu Asn Pro 5 10 55 Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys

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Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Cys Lys Lys Thr Met Leu 35 40 45

Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser 5 Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser 10 85

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30 Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Cys Lys Thr Met Leu 35

35 Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser

Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr 40

Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser 85

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50 <213> Artificial Sequence

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60 Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys 20 30

Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Lys Cys Thr Met Leu 5 Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser 10 Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr 70 75 Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser 15 85 <210> 19 <211> 92 20 <212> PRT <213> Artificial Sequence <220> <223> hCG alpha subunit with Cys substituted for Thr46 25 <400> 19 Ala Pro Asp Val Gln Asp Cys Pro Glu Cys Thr Leu Gln Glu Asn Pro 5 10 30 Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys 1 35 Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Lys Lys Cys Met Leu 40 Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr 45 Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser 85 50 <210> 20 <211> 92 <212> PRT 55 <213> Artificial Sequence <220> <223> hCG alpha-subunit with Cys substituted for Met47 60 <400> 20 Ala Pro Asp Val Gln Asp Cys Pro Glu Cys Thr Leu Gln Glu Asn Pro

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	Phe	Ser	Arg 35	Ala	Tyr	Pro	Thr	Pro 40	Leu	Arg	Ser	Lys	Lys 45	Thr	Cys	Leu
10	Val	Gln 50	Lys	Asn	Val	Thr	Ser 55	Glu	Ser	Thr	Cys	Сув 60	Val	Ala	Lys	Ser
15	Tyr 65	Asn	Arg	Val	Thr	Val 70	Met	Gly	Gly	Phe	Lys 75	Val	Glu	Asn	His	Thr 80
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40	Phe	Ser	Arg 35	Ala	Tyr	Pro	Thr	Pro 40	Leu	Arg	Ser	Lys	Lys 45	Thr	Met	Cys
45	Val	Gln 50	Lys	Asn	Val	Thr	Ser 55	Glu	Ser	Thr	Cys	Cys 60	Val	Ala	Lys	Ser
50	Tyr 65	Asn	Arg	Val	Thr	Val 70	Met	Gly	Gly	Phe	Lys 75	Val	Glu	Asn	His	Thr 80
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Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys

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Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Lys Lys Thr Met Leu

15

Cys Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser 60 50

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Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr

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Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser

<210> 23

<211> 92

<212> PRT

<213> Artificial Sequence

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<400> 23

Ala Pro Asp Val Gln Asp Cys Pro Glu Cys Thr Leu Gln Glu Asn Pro 10

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Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys. 25

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Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Lys Lys Thr Met Leu 40

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Val Cys Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser 50

Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr 55 75 65 70

Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser

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Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser

85 90

<213> Artificial Sequence

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10 <223> hCG alpha-subunit with Cys substituted for Val53

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Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys
20 25 30

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Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Lys Lys Thr Met Leu 35 40 45

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Val Gln Lys Asn Cys Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser 50 55 60

Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr 65 70 75 80

Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser 35 85 90

<210> 27

<211> 92

<212> PRT

<213> Artificial Sequence

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<223> hCG alpha-subunit with Cys substituted for Glu56

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<400> 27

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Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys 20 25 30

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Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Lys Lys Thr Met Leu 35 40 45

60 Val Gln Lys Asn Val Thr Ser Cys Ser Thr Cys Cys Val Ala Lys Ser 50 55 60

Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr

5 Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser

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15 <223> hCG alpha-subunit with Cys substituted for Ser64

<400> 28

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Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Lys Lys Thr Met Leu 35

Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Cys Ser 55

35 Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr 75

Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser 40

<210> 29

<211> 92

<212> PRT

<213> Artificial Sequence

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<223> hCG alpha-subunit with Cys substituted for Val76

<400> 29

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Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys

Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Lys Lys Thr Met Leu 40 35 45

Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser 55 5 Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Cys Glu Asn His Thr 70 10 Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser 85 <210> 30 15 <211> 92 <212> PRT <213> Artificial Sequence <220> 20 <223> hCG alpha-subunit with Cys substituted for Thr86 <400> 30 Ala Pro Asp Val Gln Asp Cys Pro Glu Cys Thr Leu Gln Glu Asn Pro 25 Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys 30 Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Lys Lys Thr Met Leu 35 Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser 60 40 Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr 70 75 65 Ala Cys His Cys Ser Cys Cys Tyr Tyr His Lys Ser 45 85 <210> 31 <211> 92 50 <212> PRT <213> Artificial Sequence <223> hCG alpha-subunit with Cys substituted for Tyr88 55 <400> 31 Ala Pro Asp Val Gln Asp Cys Pro Glu Cys Thr Leu Gln Glu Asn Pro 60

Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys 20 25 30

Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Lys Lys Thr Met Leu 5 Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser 10 Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr 15 Ala Cys His Cys Ser Thr Cys Cys Tyr His Lys Ser 85 <210> 32 20 <211> 92 <212> PRT <213> Artificial Sequence <220> 25 <223> hCG alpha-subunit with Cys substituted for Leu89 <400> 32 Ala Pro Asp Val Gln Asp Cys Pro Glu Cys Thr Leu Gln Glu Asn Pro 30 5 10 Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys 20 25 35 Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Lys Lys Thr Met Leu 35 45 40 Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser 50 55 60 45 Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr 65 Ala Cys His Cys Ser Thr Cys Tyr Cys His Lys Ser 50 <210> 33 <211> 92 55 <212> PRT <213> Artificial Sequence <220> <223> hCG alpha-subunit with Cys substituted for His90 60 <400> 33

17/41

Ala Pro Asp Val Gln Asp Cys Pro Glu Cys Thr Leu Gln Glu Asn Pro

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15	Phe	Ser	Arg 35	Ala	Tyr	Pro	Thr	Pro 40	Leu	Arg	Ser	Lys	Lys 45	Thr	Met	Leu
	Val	Gln 50	Lys	Asn	Val	Thr	Ser 55	Glu	Ser	Thr	Cys	Суs 60	Val	Ala	Lys	Ser
20	Tyr 65	Asn	Arg	Val	Thr	Val 70	Met	Gly	Gly	Phe	Lys 75	Val	Glu	Asn	His	Thi
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55	Ser	Tyr	Ala	Val	Ala	Leu	ser	Сув	Gln	Cys	Ala	Leu	Cys	Arg	Arg	Sei

Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu Thr Cys Asp Asp 100 105 110

60

Pro Arg Phe Gln Asp Ser Ser Ser Ser Lys Ala Pro Pro Pro Ser Leu 115

5 Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln 135

<210> 37 10

<211> 145

<212> PRT

<213> Artificial Sequence

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15 <223> hCG beta-subunit with Cys substituted for Ser138

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Ala Val Glu Lys Glu Gly Cys Pro Val Cys Ile Thr Val Asn Thr Thr 20 25

Ile Cys Ala Gly Tyr Cys Pro Thr Met Thr Arg Val Leu Gln Gly Val 35 40

30 Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg Asp Val Arg Phe 50 55 60

35 Glu Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Val Asn Pro Val Val 70 75

Ser Tyr Ala Val Ala Leu Ser Cys Gln Cys Ala Leu Cys Arg Arg Ser 40 90

Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu Thr Cys Asp Asp

Pro Arg Phe Gln Asp Ser Ser Ser Ser Lys Ala Pro Pro Pro Ser Leu 120

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Ala Val Glu Lys Glu Gly Cys Pro Val Cys Ile Thr Val Asn Thr Thr 20 25 30

Ile Cys Ala Gly Tyr Cys Pro Thr Met Thr Arg Val Leu Gln Gly Val

Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg Asp Val Arg Phe
50 55 60

20 Glu Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Val Pro Asn Val Val 65 70 75 80

Ser Tyr Ala Val Ala Leu Ser Cys Gln Cys Ala Leu Cys Arg Arg Ser 25 85 90 95

Thr Thr Asp Cys Thr Val Arg Gly Leu Gly Pro Ser Tyr Cys Ser Phe 100 105 110

Gly Glu Phe Gln Asp Ser Ser Ser Ser Lys Ala Pro Pro Pro Ser Leu 115 120 125

Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln
130 135 140

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Ile Cys Ala Gly Tyr Cys Pro Thr Met Thr Arg Val Leu Gln Gly Val
35 40 45

5	Leu	Pro 50	Ala	Leu	Pro	Gln	Val 55	Val	Сув	Asn	Tyr	Arg 60	Asp	Val	Arg	Phe	
	Glu 65	Ser	Ile	Arg	Leu	Pro 70	Gly	Cys	Pro	Arg	Gly 75	Val	Pro	Asn	Val	Val 80	
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	Cys	Gly	Phe	Сув 20	Ile	Thr	Ile	Asn	Thr 25	Thr	Trp	Сув	Ala	Gly 30	Tyr	Сув	
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55	Gln	Cys	His	Cys	Gly 85	Lys	Cys	Asp	Ser	Asp 90	Ser	Thr	Asp	Суз	Thr 95	Val	
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20	Tyr	Thi	Arg 35	Asp	Leu	Val	Tyr	Lys 40	Asp	Pro	Ala	Arg	Pro 45	Lys	Ile	Gln
,	Lys	Thi	с Сув	Thr	Phe	Lys	Glu 55	Leu	Val	Tyr	Glu	Thr 60	Val	Arg	Val	Pro
25	Gly 65	Суя	s Ala	His	His	Ala 70	Asp	Ser	Leu	Tyr	Thr 75	Tyr	Pro	Val	Ala	Thr 80
30	Gln	Cys	His	Cys	Gly 85	Lys	Cys	Asp	Ser	Asp 90	Ser	Thr	Asp	Cys	Thr 95	Val
35	Arg	Glλ	, Leu	Gly 100	Pro	Ser	Tyr	Cys	Ser 105	Phe	Gly	Glu	Phe	Gln 110	Asp	Ser
40	Ser	Sei	Ser 115	Lys	Ala	Pro	Pro	Pro 120	Ser	Leu	Pro	Ser	Pro 125	Ser	Arg	Leu
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	Cys	Gly	Phe	Cys	Ile	Thr	Ile	Asn	Thr 25	Thr	Trp	Сув	Ala	Gly 30	Tyr	Cys

5	Tyr	Thr	Arg 35	Asp	Leu	Val	Tyr	Lys 40	Asp	Pro	Ala	Arg	Pro 45	Lys	Ile	Gln
	Lys	Thr 50	Сув	Thr	Phe	Lys	Glu 55	Leu	Val	Tyr	Glu	Thr 60	Val	Arg	Val	Pro
10	Gly 65	Сув	Ala	His	His	Ala 70	Asp	Ser	Leu	Tyr	Thr 75	Tyr	Pro	Val	Ala	Thr 80
15	Gln	Сув	His	Cys	Gly 85	Lys	Cys	Asp	Ser	Asp 90	Ser	Thr	Asp	Cys	Thr 95	Val
20	Arg	Gly	Leu	Gly 100	Pro	Ser	Tyr	Сув	Ser 105	Phe	Gly	Glu	Phe	Gln 110	Asp	Ser
25	Ser	Ser	Ser 115	Lys	Ala	Pro	Pro	Pro 120	Ser	Leu	Pro	Ser	Pro 125	Ser	Arg	Leu
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45			-													
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50	Ile	Сув	Ala 35	Gly	Tyr	Суз	Pro	Thr 40	Met	Thr	Arg	Val	Leu 45	Gln	Gly	Val
55	Leu	Pro 50	Ala	Leu	Pro	Gln	Val 55	Val	Cys	Asn	Tyr	Arg 60	Asp	Val	Arg	Phe
60	Glu 65	Ser	Ile	Arg	Leu	Pro 70	Gly	Cys	Pro	Arg	Gly 75	Val	Asn	Pro	Val	Val 80
	Ser	Tyr	Ala	Val	Ala 85	Leu	Ser	Cys	Gln	Cys 90	Ala	Leu	Cys	Arg	Arg 95	Ser

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	Pro	Arg	Phe 115	Gln	Asp	Ser	Ser	Ser 120	Ser	Lys	Ala	Pro	Pro 125	Pro	Ser	Leu
10	Pro	Ser 130	Pro	Ser	Arg	Leu	Pro 135	Gly	Pro	Cys	Asp	His 140	Pro	Glu	Thr	Leu
15	Val 145	Lys	Val	Lys	Asp	Ala 150	Glu	Asp	Gln	Leu	Gly 155	Ala	Arg	Val	Gly	Tyr 160
20	Ile	Glu	Leu	Asp	Leu 165	Asn	Ser	Gly	Lys	Ile 170	Leu	Glu	Ser	Phe	Arg 175	Pro
25	Glu	Glu	Arg	Phe 180	Pro	Met	Met	Ser	Thr 185	Phe	Lys	Val	Leu	Leu 190	Cys	Gly
	Ala	Val	Leu 195	Ser	Arg	Ile	Asp	Ala 200	Gly	Gln	Glu	Gln	Leu 205	Gly	Arg	Arg
30	Ile	His 210	Tyr	Ser	Gln	Asn	Asp 215	Leu	Val	Glu	Tyr	Ser 220	Pro	Val	Thr	Glu
35	Lys 225	His	Leu	Thr	Asp	Gly 230	Met	Thr	Val	Arg	Glu 235	Leu	Сув	Ser	Ala	Ala 240
40	Ile	Thr	Met	Ser	Asp 245	Asn	Thr	Ala	Ala	Asn 250	Leu	Leu	Leu	Thr	Thr 255	Ile
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	Val	Thr	Arg 275	Leu	Asp	Arg	Trp	Glu 280	Pro	Glu	Leu	Asn	Glu 285	Ala	Ile	Pro
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	Ala	Leu	Pro	Ala 340	Gly	Trp	Phe	Ile	Ala 345	Asp	Lys	Ser	Gly	Ala 350	Gly	Glu

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40	Ile	Cys	Ala 35	Gly	Tyr	Сув	Pro	Thr 40	Met	Thr	Arg	Val	Leu 45	Gln	Gly	Val
	Leu	Pro 50	Ala	Leu	Pro		Val 55	Val	Сув	Asn		Arg 60	Asp	Val	Arg	Phe
45 .	Glu 65	Ser	Ile	Arg	Leu	Pro 70	Gly	Cys	Pro	Arg	Gly 75	Val	Asn	Pro	Val	Val 80
50	Ser	Tyr	Ala	Val	Ala 85	Leu	Ser	Cys	Gln	Суs 90	Ala	Leu	Cys	Arg	Arg 95	Ser
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60	Pro	Arg	Phe 115	Gln	Asp	Ser	Ser	Ser 120	Ser	ГÀв	Ala	Pro	Pro 125	Pro	Ser	Leu
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	Gly	Ala	Arg	Val	Gly 165	Tyr	Ile	Glu	Leu	Asp 170	Leu	Asn	Ser	Gly	Lys 175	Ile
10	Leu	Glu	Ser	Phe 180	Arg	Pro	Glu	Glu	Arg 185	Phe	Pro	Met	Met	Ser 190	Thr	Phe
15	Lys	Val	Leu 195	Leu	Суз	Gly	Ala	Val 200	Leu	Ser	Arg	Ile	Asp 205	Ala	Gly	Gln
20	Glu	Gln 210	Leu	Gly	Arg	Arg	Ile 215	His	Tyr	Ser	Gln	Asn 220	Asp	Leu	Val	Glu
25	Tyr 225	Ser	Pro	Val	Thr	Glu 230	Lys	His	Leu	Thr	Asp 235	Gly	Met	Thr	Val	Arg 240
	Glu	Leu	Сув	Ser	Ala 245	Ala	Ile	Thr	Met	Ser 250	Asp	Asn	Thr	Ala	Ala 255	Asn
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35	His	Asn	Met 275	Gly	Asp	His	Val	Thr 280	Arg	Leu	Asp	Arg	Trp 285	Glu	Pro	Glu
40	Leu	Asn 290	Glu	Ala	Ile	Pro	Asn 295	Asp	Glu	Arg	Asp	Thr 300	Thr	Met	Pro	Val
45	Ala 305	Met	Ala	Thr	Thr	Leu 310	Arg	Lys	Leu	Leu	Thr 315	Gly	Glu	Leu	Leu	Thr 320
.•	Leu	Ala	Ser	Arg	Gln 325	Gln	Leu	Ile	Asp	Trp 330	Met	Glu	Ala	Asp	Lys 335	Val
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60	Leu	Gly 370	Pro	Asp	Gly	Lys	Pro 375	Ser	Arg	Ile	Val	Val 380	Ile	Tyr	Thr	Thr
	Gly	Ser	Gln	Ala	Thr	Met	Asp	Glu	Arg	Asn	Arg	Gln	Ile	Ala	Glu	Ile

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Gly Ala Ser Leu Ile Lys His Trp 405

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Ala Val Glu Lys Glu Gly Cys Pro Val Cys Ile Thr Val Asn Thr Thr
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Ile Cys Ala Gly Tyr Cys Pro Thr Met Thr Arg Val Leu Gln Gly Val 35 40 45

30 Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg Asp Val Arg Phe 50 55 60

Glu Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Val Asn Pro Val Val 35 65 70 75 80

Ser Tyr Ala Val Ala Leu Ser Cys Gln Cys Ala Leu Cys Arg Arg Ser 85 90 95

Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu Thr Cys Asp Asp 100 105 110

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Pro Arg Phe Gly Pro Cys Asp Thr Pro Ile Leu Pro Gln 115 120

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<211> 130

<212> PRT

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Ala Val Glu Lys Glu Gly Cys Pro Val Cys Ile Thr Val Asn Thr Thr  $20 \hspace{1cm} 25 \hspace{1cm} 30$ 

- 5 Ile Cys Ala Gly Tyr Cys Pro Thr Met Thr Arg Val Leu Gln Gly Val
  35 40 45
- Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg Asp Val Arg Phe
  50 55 60
- Glu Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Val Asn Pro Val Val 65 70 75 80
  - Ser Tyr Ala Val Ala Leu Ser Cys Gln Cys Ala Leu Cys Arg Arg Ser 85 90 95
- 20
  Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu Thr Cys Asp Asp 100 105 110
- 25 Pro Arg Phe Gln Asp Ser Ser Ser Gly Pro Cys Asp Thr Pro Ile Leu 115 120 125
- Pro Gln
- <210> 47

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- <213> Artificial Sequence
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  - Ala Val Glu Lys Glu Gly Cys Pro Val Cys Ile Thr Val Asn Thr Thr
- 50
  Ile Cys Ala Gly Tyr Cys Pro Thr Met Thr Arg Val Leu Gln Gly Val
  35
  40
  45
- 55 Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg Asp Val Arg Phe 50 55 60
- Glu Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Val Asn Pro Val Val 60 65 70 75 80
  - Ser Tyr Ala Val Ala Leu Ser Cys Gln Cys Ala Leu Cys Arg Arg Ser

85 90 95

Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu Thr Cys Asp Asp 5 100 105 110

Pro Arg Phe Gln Asp Ser Ser Ser Ser Lys Ala Pro Pro Pro Gly Pro 115 120 125

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Cys Asp Thr Pro Ile Leu Pro Gln 130 135

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Ile Cys Ala Gly Tyr Cys Pro Thr Met Thr Arg Val Leu Gln Gly Val 35 40 45

Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg Asp Val Arg Phe 50 60 40

Glu Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Val Asn Pro Val Val 65 70 75 80

Ser Tyr Ala Val Ala Leu Ser Cys Gln Cys Ala Leu Cys Arg Arg Ser 85 90 95

50 Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu Thr Cys Asp Asp 100 105 110

Pro Arg Phe Gln Asp Ser Ser Ser Lys Ala Pro Pro Pro Ser Leu 115 120 125

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<210> 49 <211> 92

<212> PRT <213> Art:

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<400> 49

Ala Pro Asp Val Gln Asp Cys Pro Glu Cys Thr Leu Gln Glu Asn Pro 10 1 5 10 15

Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys 20 25 30

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Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Lys Lys Thr Met Leu 35 40 45

20

Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser 50 55 60

25 Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr 65 70 75 80

Ala Cys His Cys Ser Thr Cys Tyr Tyr His Glu Ser 30 85 90

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<211> 92

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<223> hCG alpha-subunit loop 2, Lys91 replaced with Met

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Ala Pro Asp Val Gln Asp Cys Pro Glu Cys Thr Leu Gln Glu Asn Pro 1 5 10 15

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Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys 20 25 30

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Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Lys Lys Thr Met Leu 35 40 45

55 Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser 50 55 60

Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr 60 65 70 75 80

Ala Cys His Cys Ser Thr Cys Tyr Tyr His Met Ser

85 90

<210> 51 <211> 92 <212> PRT <213> Artificial Sequence 10 <223> hCG alpha-subunit loop 2, Lys44 replaced with Ala <400> 51 Ala Pro Asp Val Gln Asp Cys Pro Glu Cys Thr Leu Gln Glu Asn Pro 15 5 10 Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys 20 Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Ala Lys Thr Met Leu 25 Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser 30 Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser 35 85 <210> 52 <211> 92 40 <212> PRT <213> Artificial Sequence <220> <223> hCG alpha-subunit loop 2, Lys44 replaced with Glu and Lys45 repla 45 ced with Gln <400> 52 Ala Pro Asp Val Gln Asp Cys Pro Glu Cys Thr Leu Gln Glu Asn Pro 50 Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys 55

Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Glu Gln Thr Met Leu 35 40 45

Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser 50 55 60

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Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr 65 70 75 80

Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser

85 90

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Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys 25 20 25 30

Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Arg Lys Thr Met Leu 35 40 45

Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser 50 55 60

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Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr
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75
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40 Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser 85 90

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1 5 10 15

60 Phe Gln Asp Ser Ser Ser Ser Lys Ala Pro Pro Pro Ser Leu Pro Ser 20 25 30

Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln Ala

- 5 Pro Asp Val Gln Asp Cys Pro Glu Cys Thr Leu Gln Glu Asn Pro Phe
- Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys Phe 10
- Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Lys Lys Thr Met Leu Val 15

Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser Tyr 100 105 110

20 Asn Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr Ala 120 115

- 25 Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser 135
- <210> 55 30 <211> 31 <212> PRT <213> Homo sapiens

<400> 55 35

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<400> 56

Phe Gln Asp Ser Ser Ser Ser Lys Ala Pro Pro Pro Ser Leu Pro Ser 10

- 40 Pro Ser Arg Leu Pro Gly Pro Ser Thr Asp Pro Ile Leu Pro Gly 25 20
- <210> 56 45 <211> 10 <212> PRT
  - <213> Artificial Sequence
- <220> 50 <223> Xl-Asp-Asp-Asp-Lys-Ser-Ym-Cys-Zn, where X, Y, and Z refer to any tail portion amino acids and 1, m, and n refer to the lengths of the tail portion amino acids
- <220> 55 <221> MISC FEATURE Xaa refers to any tail portion amino acids and n refers to the <223>
- lengths of the tail portion amino acids

Xaan Asp Asp Asp Asp Lys Ser Xaan Cys Xaan

5 10

5 <210> 57 <211> 92 <212> PRT <213> Artifical Sequence 10 <220> <223> An hCG truncated β-subunit analog fused to the hCG alpha-carboxyterminus <400> 57 15 Ala Pro Asp Val Gln Asp Cys Pro Glu Cys Thr Leu Gln Glu Asn Pro ' Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys 20 Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Lys Lys Thr Met Leu Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser 25 Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr 30 Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser Asp Asp Pro Arg Phe Gly Pro Cys Asp Thr Pro Ile Leu Pro Gln 35 ₹210> 58 <211> 145 <212> PRT

<213> Artificial Sequence

<220>

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<223> hCG beta-subunit with Cys substituted for Arg94

<400> 58

Ser Lys Glu Pro Leu Arg Pro Arg Cys Arg Pro Ile Asn Ala Thr Leu 10

50 Ala Val Glu Lys Glu Gly Cys Pro Val Cys Ile Thr Val Asn Thr Thr

Ile Cys Ala Gly Tyr Cys Pro Thr Met Thr Arg Val Leu Gln Gly Val 55

Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg Asp Val Arg Phe

60

Glu Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Val Pro Asn Val Val
65 70 75 80

- 5 Ser Tyr Ala Val Ala Leu Ser Cys Gln Cys Ala Leu Cys Cys Arg Ser 85 90 95
- Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu Thr Cys Asp Asp 10 100 105 110
- Pro Arg Phe Gln Asp Ser Ser Ser Ser Lys Ala Pro Pro Pro Ser Leu 115 120 125
  - Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln 130 135 140

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- <210> 59
- <211> 145
- <212> PRT
- <213> Artificial Sequence

<220>

- <223> hCG beta-subunit with Cys substituted for Arg95
- <400> 59
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- 35 Ala Val Glu Lys Glu Gly Cys Pro Val Cys Ile Thr Val Asn Thr Thr 20 25 30
- Ile Cys Ala Gly Tyr Cys Pro Thr Met Thr Arg Val Leu Gln Gly Val 40 35 40 45
- Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg Asp Val Arg Phe
  50 55 60
  - Glu Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Val Pro Asn Val Val 65 70 75 80
- Ser Tyr Ala Val Ala Leu Ser Cys Gln Cys Ala Leu Cys Arg Cys Ser 85 90 95
- 55 Thr Thr Asp Cys Gly Gly Pro Lys Asp His Pro Leu Thr Cys Asp Asp 100 105 110
- Pro Arg Phe Gln Asp Ser Ser Ser Lys Ala Pro Pro Pro Ser Leu 115 120 125

Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln
130 135 140

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	Ser 1	ГÀЗ	Glu	Pro	Leu 5	Arg	Pro	Arg	Cys	Arg 10	Pro	Ile	Asn	Ala	Thr 15	Leu	
5	Ala	Val	Glu	Lys 20	Glu	Gly	Cys	Pro	Val 25	Cys	Ile	Thr	Val	Asn 30	Thr	Thr	
10	Ile	Сув	Ala 35	Gly	Tyr	Сув	Pro	Thr 40	Met	Thr	Arg	Val	Leu 45	Gln	Gly	Val	
15	Leu	Pro 50	Ala	Leu	Pro	Gln	Val 55	Val	Cys	Asn	Tyr	Arg 60	Asp	Val	Arg	Phe	
	Glu 65	Ser	Ile	Arg	Leu	Pro 70	Gly	Cys	Pro	Arg	Gly 75	Val	Pro	Asn	Val	Val 80	
20	Ser	Tyr	Ala	Val	Ala 85	Leu	Ser	Сув	Gln	Сув 90	Alá	Leu	Сув	Arg	Arg 95	Ser	
25	Cys	Thr	Asp	Cys 100	Gly	Gly	Pro	Lys	Asp 105	His	Pro	Leu	Thr	Cys 110	Asp	Asp	
30	Pro	Arg	Phe 115	Gln	Asp	Ser	Ser	Ser 120	Ser	Lys	Ala	Pro	Pro 125	Pro	Ser	Leu	
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55	Ile	Cys	Ala 35	Gly	Tyr	Cys	Pro	Thr 40	Met	Thr	Arg	Val	Leu 45	Gln	Gly	Val	
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Glu Ser Ile Arg Leu Pro Gly Cys Pro Ara Gly Val Pro Asn Val Val

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	65	70	75	80
5	Ser Tyr Ala Val Ala 85	Leu Ser Cys Gln Cys 90	Ala Leu Cys Arg Arg 95	Ser
10	Thr Cys Asp Cys Gly 100	Gly Pro Lys Asp His 105	Pro Leu Thr Cys Asp	Asp
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35	Ala Val Glu Lys Glu 20	Gly Cys Pro Val Cys 25	Ile Thr Val Asn Thr	Thr
	Ile Cys Ala Gly Tyr 35	Cys Pro Thr Met Thr 40	Arg Val Leu Gln Gly 45	Val
40	Leu Pro Ala Leu Pro 50	Gln Val Val Cys Asn 55	Tyr Arg Asp Val Arg	Phe
45	Glu Ser Ile Arg Leu 65	Pro Gly Cys Pro Arg	Gly Val Pro Asn Val	. Val 80
50	Ser Tyr Ala Val Ala . 85	Leu Ser Cys Gln Cys 90	Ala Leu Cys Arg Arg 95	ser
55	Thr Thr Cys Cys Gly 100	Gly Pro Lys Asp His 105	Pro Leu Thr Cys Asp 110	Asp
	Pro Arg Phe Gln Asp 115	Ser Ser Ser Ser Lys 120	Ala Pro Pro Pro Ser 125	Leu
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140

135

130

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     Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Lys Lys Thr Met Leu
20
     Val Gln Lys Asn Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser
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     Ser92
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     Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln Cys Met Gly Cys Cys
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     Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser Lys Lys Thr Met Leu
50
     Val Gln Lys Asp Val Thr Ser Glu Ser Thr Cys Cys Val Ala Lys Ser
     Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys Val Glu Asn His Thr
55
     Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser
                     87
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<210> 66 <211> 145 <212> PRT

<213> Artificial Sequence

5 <220>

15

<223> hCG beta-subunit with Cys substituted for Ser96 and hFSH beta-subunit residues 95-108 for hCG beta-subunit residues 101-108

- 10 <400> 66
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- Ala Val Glu Lys Glu Gly Cys Pro Val Cys Ile Thr Val Asn Thr Thr 20 25 30
- 20 Ile Cys Ala Gly Tyr Cys Pro Thr Met Thr Arg Val Leu Gln Gly Val 35 40 45
- Leu Pro Ala Leu Pro Gln Val Val Cys Asn Tyr Arg Asp Val Arg Phe 50 50 55 60
- Glu Ser Ile Arg Leu Pro Gly Cys Pro Arg Gly Val Pro Asn Val Val 65 70 75 80
  - Ser Tyr Ala Val Ala Leu Ser Cys Gln Cys Ala Leu Cys Arg Arg Cys 85 90 95
- Thr Thr Asp Cys Thr Val Arg Gly Leu Gly Pro Ser Tyr Cys Ser Phe
  100 105 110
- 40 Gly Glu Phe Gln Asp Ser Ser Ser Ser Lys Ala Pro Pro Pro Ser Leu
  115 120 125
- Pro Ser Pro Ser Arg Leu Pro Gly Pro Ser Asp Thr Pro Ile Leu Pro Gln 130 135 140